QUALITY OF RABBIT MEAT UNDER MODIFIED ATMOSPHERES

Berruga, M.I. [1,2], Vergara, H[1,2]. and Linares, M.B. [1,2]

[1]Departamento de Ciencia y Tecnología Agroforestal, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, 02071 Albacete, Spain

[2]Sección de Calidad Alimentaria, Instituto de Desarrollo Regional, Universidad de Castilla-La Mancha, 02071 Albacete, Spain

Background

Meat from rabbit is one of the healthiest meats because its dietetics properties (Dalle Zotte, 2002), which make it recommendable by the WHO to children, older and unhealthy people nutrition. In spite of these characteristics, rabbit meat consumption is very low (less than 3 kg/person in Europe) and is therefore in need of a publicity campaign and appropriate distribution channels. Distribution could be improved by conservation in modified atmospheres (MA) in order to prolong shelf-life for a time longer than normal refrigeration allows.

Only the effect of CO_2 and N_2 atmospheres (Gariepy *et al*., 1986), and vacuum (Fernández-Esplá and O'Neil, 1993) have been evaluated in rabbit meat, and a lack of studies on modified atmospheres packaging for fresh rabbit meat is perceived (Dalle Zotte, 2002).

Objectives

The aim of this study was to evaluate some parameters of rabbit meat when is packed in modified atmospheres with different gas compositions, to suggest a package atmosphere that best preserves the initial characteristics of rabbit meat and would have better acceptation.

Materials and methods

Sixty-eight rabbits from the Cunicultura-Villamalea S.A. Company (Albacete, Spain) were selected and slaughtered using standard commercial procedures. Carcasses were chilled at 1ºC for a day, and after that were cut in half-carcasses.

One hundred and thirty-six half-carcasses (550 g), without liver, were grouped in 8 half-carcasses to study initial meat quality (1 d post-slaughter), and 128 half-carcasses to study meat quality evolution under MA.

Samples were randomly assigned (32 half-carcasses per MA) to one of the following four types of MA: *Atmosphere A*: 30% CO_2 + 70% O_2; *Atmosphere B*: 30% CO_2 + 30% O_2 + 40% N_2; *Atmosphere C*: 40% CO_2 + 60% N_2; and A*tmosphere D*: 80% CO_2 + 20% O_2

A thermoforming atmosphere packaging machine ULMA Packaging model Smart (Guipuzcoa, Spain) was used for packing. The samples were places in clear rigid trays (LINPAC Plastic), having an oxygen permeability (OP) rate of 3.2 cm^3 /m^2 /24 h at 1 atm and 23°C ,and a cover film with an OP of 4.1 cm^3 /m^2 /24 h at 1 atm and 25°C) with the correspondent gas mixture.

Samples were chilled at 1°C for up to 21 days post-slaughter (i.e. 5, 10, 15 and 20 post-packing).

Eight samples were examined per atmosphere type (A, B, C, D) and per sampling time to determinate off-odour, instrumental and sensorial colour and rancidity evolution on meat.

Sample rancidity was determined in duplicate from 2 g of the *Longisimuss dorsi* (LD) muscle as described by Botsoglou *et al.* (1994) by determining 2-thiobarbituric acid-reactive sustances (TBARS). Absorbencies were measured with a spectrophotometer Perkin Elmer Lambda 20 (Norkwalk, USA) at 532 nm. Results were expressed as mg malondialdehyde Kg^{-1} meat.

Colour measurements (*L*, a*, b**) were taken immediately after that the packs were opened and the measurements were taken on the surface of the LD, at the level of the fourth lumbar vertebra. Measurements were recorded using a Minolta CR200 colorimeter (Osaka, Japan) calibrated against a standard white tile.

Sensorial colour and odour were also assessed by a panel of five experts. Colour was categorised in closed packs, and odour inmediatly after opened its. For both colour and odour, samples were scored as 1= not acceptable, 2= acceptable or 3= very acceptable.

The data were analysed using analysis of variance to determine the effects of atmosphere type on colour and rancidity. When the differences among types of modified atmospheres were significant ($p< 0.05$), Tukey's test was carried out to check the differences between pairs of groups. The effect of time of storage in refrigeration for each MA was analysed using Tukey's test at a significance level of $p< 0.05$. Data were analysed using the SPSS 11.0.1 statistical software.

Results and discussion

Acceptability of packs according to visual evaluation was higher and longer in atmospheres type A and B (Table 1). By contrasts, samples packed in atmosphere C were not accepted at any time during this experiment due to poor colour. In agreement with Gariepy *et al.* (1986) a faster discoloration of samples stored with higher proportions of CO_2 was appreciated, which was more marked in packs without oxygen in their initial composition.

Table 1. Percentage of acceptable packs according to sensorial colour[1] and odour[2].

	Time post-packing	Type A	Type B	Type C	Type D
Colour	5 days	100	100	0	85
	10 days	100	100	0	100
	15 days	100	96	0	0
	20 days	100	50	0	0
Odour	5 days	100	100	100	97
	10 days	100	100	100	100
	15 days	100	100	100	100
	20 days	25	25	70	70

[1]Closed packs, [2]When packs were opened
Modified Atmosphere: Type A (30% CO_2 +70% O_2), Type B (30% CO_2+30% O_2 + 40% N_2), Type C (40% CO_2 + 60% N_2), and Type D (80% CO_2+20% O_2).
Samples were categorised (for both colour and odour) as 1= not acceptable (strong off-odour), 2= acceptable (slight off-odour) or 3= very acceptable (no off-odour).

Off-odour was developed faster in samples with more oxygen (Table 1). The inhibitory effects of CO_2 on the spoilage bacteria could be the cause of the better odour quality of samples containing high proportions of CO_2. Unacceptable odour has been attributed to bacterial growth over 10^6 CFU/g (Gariepy *et al.,* 1986), and after 20 days none sample from any MA presented counts higher than 10^5CFU/cm^2 (data not showed). Also Gariepy *et al.*(1986) reported no objectionable odour in rabbit carcasses packed under CO_2 for more than 20 days.

Initial values for L^*, a^* and b^* were 54.92, 3.62 and – 4.23, respectively. A significant increase in L^* values was observed in all treatments (Table 2), and this is usually attributed to the oxidation of hem-pigments (Fernández *et al.*, 2000). However there were significant differences among groups only from 15 days onwards, then the values of this parameter were higher in samples of type A (more oxygen).

Redness (coordinate a^*) increased during the first 5 days and then decreased ($p< 0.05$) in all MA treatments wiht time. Similar evolution on a^* values was reported in beef (Insausti *et al*., 2001). The decrease was more pronunced in type A, and could be the result of metmyoglobin formation (Insausti *et al*., 2001). Also, an increase in yelowness was observed in all MAs along the first 5 days. After that time, coordinate b^* took negative increasing values (more blueness), which were higher in type C the first 15 days. After that differences between b^* values were higher in samples from type D. Increases in blueness has also been reported in frozen rabbit meat by Cabanes *et al.* (1994, 1996).

Slight rancidity increases in rabbit meat were appreciated in all treatments along time (Fig 1), but the increase was only significant in atmosphere A ($p< 0.05$). After ten days of storage significant differences were appreciated among treatments in all times. The atmospheres with higher oxygen concentrations (type A and B) showed the highest rancidity rates ($p< 0.05$), and the atmosphere without oxygen (type C) the lowest ($p< 0.05$). In addition, at the end of this study malondialdehyde levels in these atmospheres (type A: 1.11 ± 0.61 mg/kg, and type B: 0.89 ± 0.51 mg/kg) were similar or inferior than those reported by other authors for refrigerated fresh rabbit meat (Corino *et al*., 1999; Castellini *et al*., 2000). According to some authors, at least 5 mg malondialdehyde/kg are required for rancidity detection (Insausti *et al.*, 2001). After 20 days the MA types A and B were inferior than this value. Studies about rancidity evolution of rabbit meat were scarce

and focused on other storage systems like vacuum packaging (Fernández-Esplá and O'Neill, 1993). As in vacuum packaging, the use of modified atmospheres with carbon dioxide allows the extension of rabbit meat shelf-life. Besides, meat samples packed in non-oxygen-MA were more stable to lipid oxidation, because the absence or the minimal residual oxygen concentrations (O'Grady *et al*., 2000; Insausti *et al*., 2001; Jeremiah, 2001).

Table 2. Values (means ± e.s.) of colour (*L, *a**, *b**) parameters of rabbit meat preserved in modified atmospheres with different gas composition.**

Colour	Time post-packing	Type A	Type B	Type C	Type D	ANOVA
*L**	5 days	64.24 ± 1.08^{a}	62.23 ± 0.82^{a}	63.35 ± 1.00^{a}	65.57 ± 0.33^{a}	NS
	10 days	67.60 ± 0.75^{ab}	66.31 ± 1.50^{ab}	67.91 ± 0.63^{bc}	65.98 ± 1.03^{a}	NS
	15 days	$69.43 \pm 0.79^{b;x}$	$64.85 \pm 1.59^{a;y}$	$66.18 \pm 0.95^{ab;xy}$	$65.53 \pm 1.04^{a;xy}$	*
	20 days	$73.89 \pm 1.13^{c;x}$	$72.13 \pm 0.49^{b;xy}$	$71.05 \pm 0.62^{c;xy}$	$70.13 \pm 1.17^{b;y}$	*
*a**	5 days	6.99 ± 1.27^{a}	7.73 ± 0.82^{a}	5.37 ± 0.51^{a}	5.29 ± 0.44^{a}	NS
	10 days	$4.16 \pm 0.44^{ab;xy}$	$6.19 \pm 1.03^{ab;y}$	$3.38 \pm 0.30^{bc;x}$	$4.93 \pm 0.59^{a;xy}$	*
	15 days	$3.58 \pm 0.48^{ab;x}$	$5.50 \pm 0.66^{ab;y}$	$4.32 \pm 0.26^{ab;xy}$	$4.44 \pm 0.32^{a;xy}$	*
	20 days	1.32 ± 0.33^{b}	3.08 ± 1.06^{b}	2.47 ± 0.53^{c}	1.79 ± 0.49^{b}	NS
*b**	5 days	$1.55 \pm 1,28^{a;x}$	$1.56 \pm 0,94^{x}$	$-2.35 \pm 0,51^{y}$	$-1.59 \pm 0,68^{xy}$	**
	10 days	$-1.97 \pm 0.35^{ab;xy}$	-0.20 ± 0.95^{x}	-3.88 ± 0.42^{y}	-1.45 ± 0.75^{xy}	**
	15 days	-1.24 ± 0.97^{ab}	-2.02 ± 1.44	-4.49 ± 0.50	-4.24 ± 0.94	NS
	20 days	$-3.17 \pm 0.35^{b;xy}$	-1.60 ± 0.94^{x}	-3.60 ± 0.08^{xy}	-5.04 ± 0.46^{y}	*

Modified Atmosphere: Type A (30% CO_2 +70% O_2), Type B (30% CO_2+30% O_2 + 40% N_2), Type C (40% CO_2 + 60% N_2), and Type D (80% CO_2+20% O_2).
*,**: indicate significance levels at 0.05 and 0.01, respectively; NS: not significant
$^{a, b, c}$: values in the same column with different superscript are significantly different ($p < 0.05$); $^{x, y}$: values in the same row with different superscript are significantly different ($p < 0.05$).

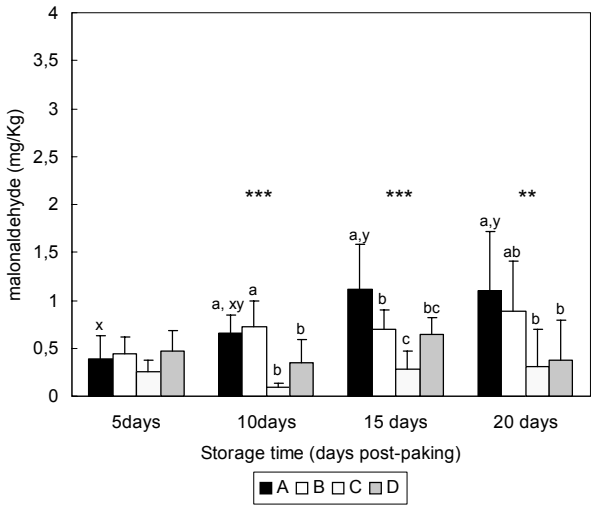


Figure 1. Rancidity levels (TBARS; mg malonaldehyde/Kg meat) in rabbit meat stored at 1°C in A: 30% CO_2 + 70% O_2 (black bars), B: 30% CO_2+30% O_2 + 40% N_2 (white bars), C: 40% CO_2 + 60% N_2 (pointed bars), and D: 80% CO_2+20% O_2 (cross bars). [**, ***: indicate significance levels at 0.01 and 0.001, respectively; $^{a, b, c}$: values in the same storage day with different superscript are significantly different ($p< 0.05$); ^{x,y}: values in the same colour bar with different superscript are significantly different ($p< 0.05$)].

Conclusions

According to colour samples from the atmosphere type A (30% CO_2 + 70% O_2) showed the best properties until the15th day; non off-odour were detected in any sample from this type, and although this atmosphere showed the highest levels of rancidity these numbers were always very inferior to the threshold level reported on the literature.

References

Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos, V.N., Mantis, A.J., and Trakatellis, A.G. 1994. Rapid, sensitive, and specific thiobarbituric acid methods for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agric. Food Chem. 42,1931-1937.

Cabanes, A., Ouhayoun, J., and Gilbert, S. 1996. Congélation de la viande de lapin. Influence de la durée de conservation sur les propriétés physico-chimiques et sensorielles (3, 6, 9, 12, 18 mois). Viandes Produits Carnés. 17: 166-171.

Cabanes-Roiron, A., Ouhayoun, J., & Gilbert, S. (1994). Qualité de la viande de lapin. Influence de trois modes de conservation sur l'évolution des propriétés microbiologiques, physico-chimiques et sensorielles. In *Proceedings of the VIèmes Journées de la Recherche Cunicole,* Vol 2 (pp. 393-402), 6-7 December 1994, La Rochelle, France.

Castellini, C., Dal Bosco, A., and Bernardini, M. 2000. Improvemente of lipid stability of rabit meat by vitamin E and C administration. J. Sci. Food Agric. 81: 46-53

Corino, C., Pastorelli, G., Pantaleo, L., Oriani, G., and Salvatori, G. 1999. Improvement of color and lipid stability of rabbit meta dietary supplementation with vitamin E. Meat Sci. 52: 285-289.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Sci. 75: 11-32.

Fernández, J., Pérez, J,A., Sayas, E., and Aranda, V. 2000. Characterization of the different sates of myoglobin in pork using color parameters and reflectance ratios. J. Muscle Food. 11: 157-168.

Fernández-Esplá, M.D., and O`Nelly, E. 1993. Lipid oxidation in rabbit meat under different storage conditions. J. Food Sci. 58: 1262-1264.

Gariepy, C., Amiot, J., Simard, R.E., Boudreau, A., and Raymond, D.P. 1986. Effect of vacuum-packing and storage in nitrogen and carbon dioxide atmospheres on the quality of fresh rabbit meat. J. Food Quality. 9: 289-309.

Insausti, K., Beriain, M.J., Purroy, A., Alberti, P., Gorraiz, C., and Alzueta, M.J. 2001. Shelf life of beef from local Spanish cattle breeds stored under modified atmosphere. Meat Sci. 57: 273-281.

Jeremiah, L.E. 2001. Packaging alternatives to deliver fresh metas using short- or long-term distribution. Food Res. Int. 34: 749-772.

O'Grady, M.N., Monahan, F.J., Burke, R.M., and Allen, P. 2000. The effect of oxygen level and exogenous alpha-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. Meat Sci. 55: 39-45.

CHANGES IN IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CALPAIN IN MUSCLE INDUCED BY CONDITIONING AND HIGH-PRESSURE TREATMENT

Gerelt Borjigin[1], Shuhei Yamamoto[2], Toshie Sugiyama[3], Tadayuki Nishiumi[2] and Atsushi Suzuki[2]

[1]Department of Applied Biological Chemistry, and [3]Department of Agricultural Science, Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan

[2]Doctoral'l Program in Biosphere Science, Graduate School of Science and Technology, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan

Key word: calpain, immunoelectron microscopy, meat tenderization, meat conditioning, high-pressure treatment

Background

The most striking effect of postmortem aging is the improvement of meat tenderness, which is almost exclusively brought about by muscle endogenous protease that disrupts myofibrils. Especially, proteolysis of key myofibrillar proteins by calpain is the underlying mechanism of meat tenderization that occurs storage of meat at refrigerated temperatures. In general, calpain is widely distributed throughout the cytoplasm in a variety of cells. The calpains are present in two distinct forms, μ-calpain, a protease requiring 3-50 μM calcium ion for half-maximal activity; m-calpain, a protease requiring 400-800 μM calcium ion for half-maximal activity. The two calpain, μ- and m-calpain, are 110-kDa molecules each containing a 80-kDa and a 28-kDa polypeptide subunit. The 28-kDa subunit is identical in μ- and m-calpain; the 80-kDa subunits from two molecules differ, although they share 50 % sequence similarity.

It is well known that high hydrostatic pressurization is one of the new technology for reducing the conditioning period and improving the meat tenderness: there are many papers describing meat tenderization or acceleration of meat conditioning due to structural changes of the myofibrils caused by high pressure (Bouton et al., 1977; Locker & Wild, 1984; Macfarlane, 1985; Suzuki et al., 1992). Regarding calpain systems, Suzuki et al. (1993) provided direct evidence for the pressure-induced Ca^{2+} release from the sarcoplasmic reticulum from electron micrographs of the pyroanthimonate-fixed fiber bundles prepared from pressurized muscles. Ca^{2+} dispersion into myofibrils may cause the increase of activated calpain. Also Homma et al. (1995) indicated that the total activities of calpain in pressurized muscle appear to have been increased by the pressure and this may result in tenderization of meat.

There have been few reports describing the changes of calpain localization in muscle by high pressure treatment, and the behavior of the enzymes has not been clear. Many histological studies suggested that the calpain in the normal muscle localized primarily to Z-disk with smaller amounts in the I-band, A-band area, and between myofibrils. However, the changes in the localization of calpain in the muscle by conditioning or pressurization were not elucidated.

Objective

The objective of this paper was to investigate the changes in the localization of calpain in muscle exposed to high pressure by using immunoelectron microscopy, in comparison with those naturally observed in conditioned muscle.

Materials and methods

Tissue preparation: Lean meat was excised from the shoulder part of a culled-cow carcass 2 days after slaughter and stored at -25℃. As required, it was tempered overnight in a cold room (3 - 4℃) and divided into two parts, one for high pressure (100-400 MPa, 5min), and the other for conditioning at 2-4℃ 7 and 14 days. Muscle samples (1 mm^3) prepared from the pressurized and conditioned muscles were fixed with 0.25 % glutaraldehyde-PBS (0.1 M phosphate, pH.7.5, 8 % sucrose) for 30 min at room temperature, washed with PBS three times. After that the samples were dehydrated in graded alcohol (50 % and 75 %), and then were embedded in LR-White resin.

Immunoelectron microscopy: Ulrtrathin sections were cut onto 300-mesh grids and were incubated for 15 min in a PBS solution containing 5 % normal goat serum. After washing with PBS, the grids were

transferred onto drop of monoclonal antibody (Mouse anti-calpain small subunit (μ - or m-calpain) monoclonal antibody) (Chemicon) for 60 min. The monoclonal antibody was diluted 1:200 with PBS buffer. After incubation with the primary antibody, the grids were washed in PBS, and colloidal gold particle 10 nm in diameter conjugated with goat anti-mouse IgG+IgM (H+L) (Amersham) was added for 30 min at room temperature. The secondary antibody was diluted 1:25 (OD at 520nm of 0.1) just before use with PBS buffer. The grids were then washed in PBS, and the specimens were stained with 2 % uranyl acetate for 5 min. The stained specimens were examined with a CM 2000 Philips electron microscope at an operating voltage of 80 kV. Control sample was incubated with PBS in place of the primary antibody followed by incubation with the secondary antibody.

Results and discussion

To confirm the specific labeling for calpain, the muscle sample prepared immediately after thawing was first treated without anti-calpain antibody, then treated with colloidal gold conjugated anti-mouse IgG + IgM (H+L) (Fig.1). In which, non-specific labeling of the muscle was not observed. When the muscle sample was stained according to the procedure described in method, the immunogold particles were observed in Z-disk, I-band and A-band regions. This is proof that the anti-calpain small subunit (μ - or m-calpain) monoclonal antibody is specific to both μ - and m-calpain. But the immunogold particle seems to be concentrated primarily to Z-disk region than either I-band or A-band regions. This result agreed with those quantitative studies (Goll et al., 1983; Ishiura et al., 1980; Kumamoto et al., 1992) that calpain is located on the Z-disk with smaller amounts in the I-band and very little in the A-band area. When muscle was examined after postmortem, the calpain distribution was altered to being more diffuse throughout the sarcomere instead of concentrated at the Z-disk and between the myofibrils. As well as high density of immunogold particle was detected throughout the sarcomere, also disruption of myofibrillar structure was observed. The localization of the calpain may be involved in myofibril degradation and in membrane alterations of muscle cell.
When the muscle was exposed to pressure of 100 MPa or 200 MPa, a little more immunogold particle was detected in the sarcomere than that of unpressurizad muscle (Fig.2). With the increasing of the pressure up to 300 MPa applied to the muscle, detected high density immunogold particle throughout the sarcomere. Inversely, a few immunogold particle was detected in the sarcomere of the muscle exposed to pressure of 400 MPa. It seems to be due to the inactivation of calpain under high pressure. Also marked disruption in the regular structure of the myofibrils was observed in the muscle pressurized at 400 MPa as compared with the conditioned muscle. This disruption seems to be not due to the proteolytic degradation of myofibrils, but mainly due to the depolymerization of the F-actin, constituents of thin filament, as suggested by Macfarlane (1985)

Conclusions

From the results obtained in this experiment, it was clear that the changes in the localization of calpain in the muscle induced by high pressure were drastic in comparison with that in the muscle during conditioning.

Acknowledgments

This work was supported by Grant-in Aid for Japan Society for the Promotion of Science (JSPS) Fellows.

References

Bouton, P. E., Ford, A. L., Harris, P. V., Macfarlane, J. J., & O'Shea, J. M. (1977). *J. Food Sci., **42,*** 132-135.

Busch, W. A., Stromer, M. H., Goll, D. E., & Suzuki, A. (1972). *J. Cell. Biol., **52,*** 367-381.

Goll, D. E., Shannon, J. D., Edmunds, T., Sathe, S. K., Kleese, W. C., & Nagainis, P. A. (1983). In: *Calcium binding proteins*, Bernard et al. (Eds.) pp.17-35. Elisevier Science Publishers, B.V., Amsterdam.

Goll, D. E., Stromer, M. H., Olson, D. G., Dayton, W. R., Suzuki, A., & Robson, R. M. (1974). In *Proc. of the Meat Industry Res. Conf.,* pp.75-97. *Am. Meat Inst. Found.* Arlington, Virginia.

Homma, N., Ikeuchi, Y., & Suzuki, A. (1995). *Meat Sci., **41,*** 251-260.

Ishiura, S., Sugita, H., Nonaka, I., & Imahori, K. (1980). *J. Biochem., **87,*** 343-346.

Kobo, T., Gerelt, B., Han, G. D., Sugiyama, T., Nishiumi, T., & Suzuki, A. (2002). *Meat Sci., **61,*** 415-418.

Kumamoto, T., Kleese, W. C., Cong. J. Y., Goll, D. E., Pierce, P. R., & Allen, R. E. (1992). *The Anato. Rec.,* ***232,*** 60-77.

Locker, R. H., & Wild, D. J. C. (1984). *Meat Sci.,* ***10,*** 207-233.

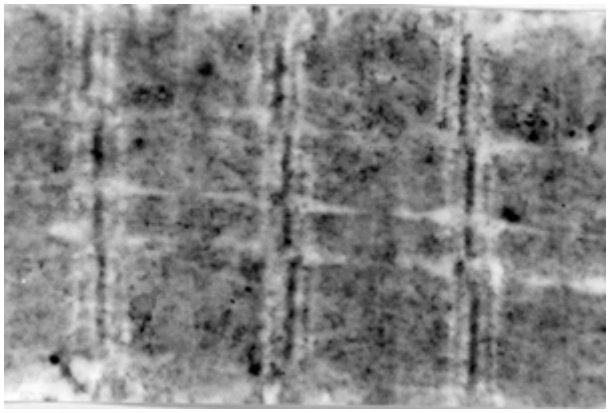
Macfarlane, J. J. (1985). In R. A. Lawrie (Ed.), *Development in meat science-3* (pp.155-184). London: Elsevier Applied Science.

Penny, I. F. (1980). In *Development in meat science*-1. (Lawrie, R. A., ed.) pp. 115-143. Elisevier Applied Science Publishers Ltd, UK.

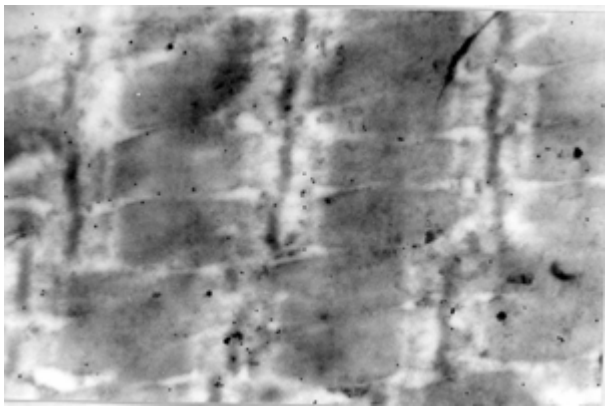
Suzuki, A. & Goll, D. E. (1974). *Agric. Biol. Chem.,* ***38,*** 2167-2175.

Suzuki, A., Kim. K., Homma, N., Ikeuchi, Y., & Saito, M. (1992). In C. Balny et al. (Eds.), *High pressure and bioscience* (pp.219-22). Montrouge: Colloques INSERM/John Libbey Eurotext.

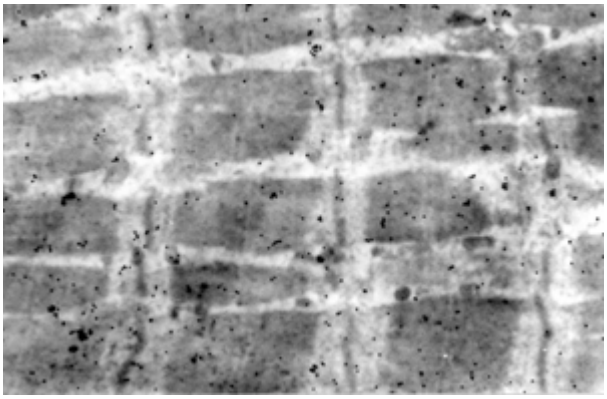
Suzuki, A., Okamoto, A., Ikeuchi, Y., & Saito, M. (1993). *Biosci. Biotech. Biochem.,* ***57,*** 862-863.



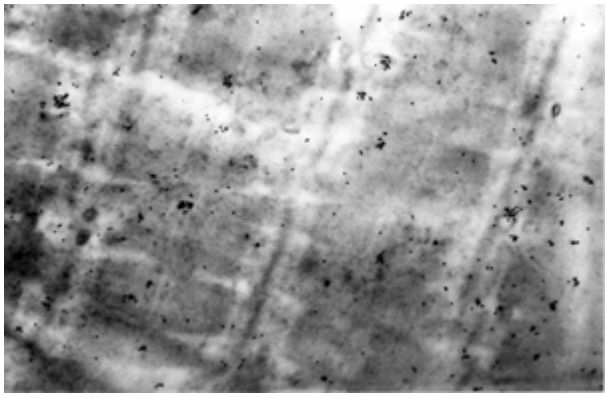
control



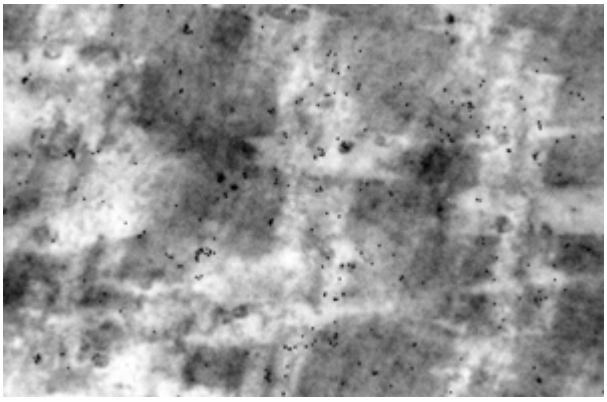
immediately after thawing



stored for 7 days

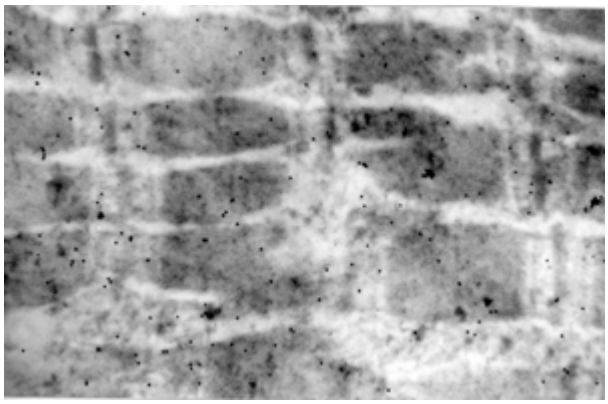


stored for 14 days

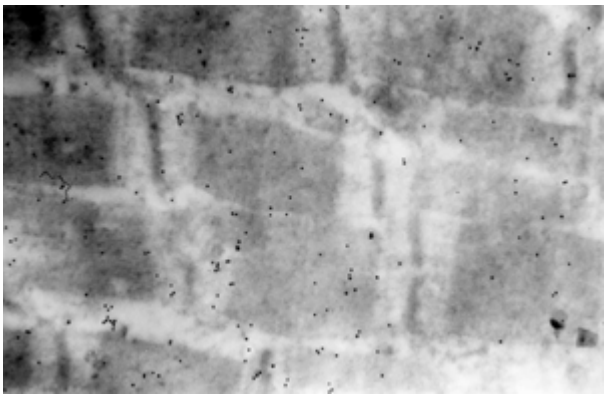


stored for 21 days

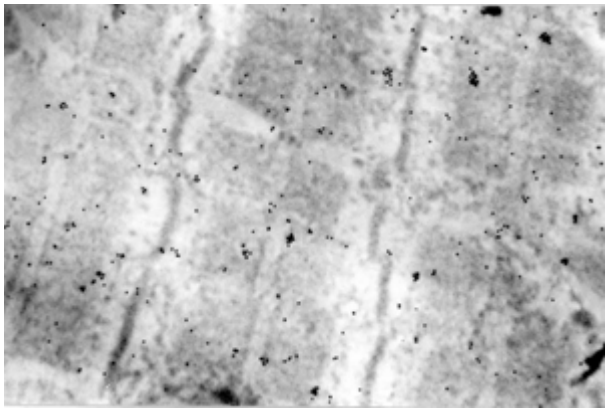
Fig.1. Immunoelectron micrographs showing localization of calpain during conditioning



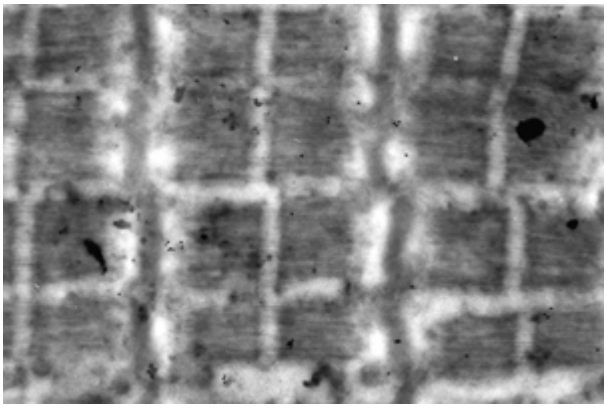
pressurized at 100 MPa



pressurized at 200 MPa



pressurized at 300 MPa



pressurized at 400 MPa

Fig.2. Immunoelectron micrographs showing the changes in localization of calpain caused by high pressure